ADVANCES IN PERCUTANEOUS ACCESS DEVICE DEVELOPMENT

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In a previous report[1] we presented hypotheses as to requirements for achieving long-term, failure-free operation of a percutaneous access device (PAD) and described the results of investigations up to that time. The overall hypothesis was, in brief, that by meeting 2 critical requirements, i.e., control of epidermal migration in proximity to the implant, and attenuation of strain at the skin-device interface, a PAD could be constructed that would tend to minimize subsequent infection, extrusion or marsupialization[2,3]. To meet these requirements we have been proceeding as follows: 1) Appropriate biomedical materials were selected for use as PAD neck material (that portion that protrudes through the skin); a nanoporous surface was formed on the PAD neck material by means of high energy nuclear particle bombardment followed by etching; 2) Autologous fibroblasts were cultivated on the nanoporous PAD neck, in vitro, stimulated to produce collagen and encouraged to form a firm, multilayered attachment over the entire surface; the resulting surface was referred to as the ALCON surface, i.e., Autologous "Living" Collagen-coated Nanoporous surface[1]; 3) A subcutaneous flange, attached to the PAD neck just prior to implantation of the complete device, was constructed so as to attach to the dermis, thereby preventing relative motion between the skin and the implant.

In this report we describe the results of further investigations of this PAD design.

MATERIALS AND METHODS

Preparation of PAD Neck Materials for the In Vitro Cultivation of Fibroblasts. After examining numerous materials for suitability in PAD neck construction[1], all but polycarbonate (Lexan 104, General Electric) and silicone polycarbonate (Copels 4020 and 3320, General Electric) were eliminated from further investigation. Lexan 104 was selected because of the relative ease with which it can be manipulated (cleaning, sterilization, sectioning) due to its rigid nature. On the other hand, the silicone polycarbonates have consistencies more closely similar to that of skin (especially Copel 4020) and thus may offer advantages as implanted PAD neck material not possible with more rigid materials.

We hypothesized that attachment of autologous cells to the PAD neck would be enhanced if micrometer-size pores at the appropriate density were present. Such pores would allow interdigitation of dermal elements and substrate, consequently increasing the contact area which should in turn increase adhesion to the biomaterial. Pores were produced by exposing the PAD necks to nuclear bombardment from a Californium-252 source in a vacuum chamber. The resulting damage tracts were etched in 6.25 N sodium hydroxide to form pores of desired dimensions[4]. PAD necks used in the experiments described below were treated to yield pores 1.0 μ in diameter and 15-20 μ in depth, at a density of 15,000/mm^2.

PAD necks were prepared for use as substrates for the cultivation of fibroblasts by first cleansing the material via sonication in a cleaning solution (Micro, International Products Corp.), followed by several rinses in glass distilled water. The materials were then sterilized using standard methods of U.V. irradiation or autoclaving.

Coating Nanoporous PAD Necks with Autologous Fibroblasts. Fibroblasts were obtained from the skin of newborn CFN rats and cultivated in vitro according to a previously reported technique[5] which results in a monolayer after 7-10 day incubation at 35°C in a 95% air and 5% CO_2 humidified atmosphere. Skin biopsy specimens from miniature swine were grown as explants which produced outgrowths of fibroblasts in 3-4 wks and a monolayer in approximately one month. The complete medium (CM) and additives used for cultivation of both rat and swine fibroblasts were described previously[1]. Fibroblast Growth Factor (FGF) and Endothelial Cell Growth Supplement (ECGS) promoted both rat and swine fibroblast proliferation in vitro. Ascorbic acid was added to induce production of crosslinked collagen. The combination of FGF, ECGS and ascorbic acid resulted in multilayered cell growth with abundant amounts of collagen deposited extracellulary (Figure 1).

A number of studies were performed to examine the nature of the relationship between fibroblast, collagen, and the biomaterial substrate. Pretreatment of samples of the biomaterials with serum, human fibronectin (HFN) or acid were compared with samples that received no such pretreatment with respect to the ability of the resulting substrates to support cellular attachment and proliferation. A total of 40 rat fibroblast cultures on Lexan 104 were studied using transmission electron microscopy (TEM).

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Studies of the Bonding of Autologous Fibroblasts to Host Dermis In Vivo. Experiments were performed in rats in vivo to study the ability of the PAD coated with autologous cells and collagen to merge with the host dermis. Two groups of experiments were initiated; the first of which has been completed. In the first experiment 28 scaled-down devices (half of them Lexan 104, half Copel 3320) were implanted into 5 CFN rats. These included smooth-surfaced controls. These rats were sacrificed 72 days after receiving implants and all 18 implants and surrounding tissues were examined histologically. One rat with 4 implants was sacrificed after 187 days and the implants examined. In the second experiment, 12 scaled-down devices were implanted into 4 rats (7 Lexan 104 and 5 Copel 4020). These also included 4 smooth surfaced controls.

In Vivo Assessment of Miniature Swine. On the basis of our studies of strain relief[1] and our earlier experience[6], a full-scale PAD was designed (Figure 2). The neck was made separately to simplify the production of the nanoporous surface and the cultivation of autologous cells on it. It was assembled to the flange at the time of implantation. To reduce the animal's discomfort and minimize the likelihood of PAD damage, implants were restricted to one side of the animal. Two sites were available for use, one on the side of the abdomen and one over the ribs.

To decrease the chances of contamination of the implantation site or savaging by the animal, a restraint system was required that protected its implanted side from injury. After trying different methods, we applied a layer of bandages around the implant sufficiently thick to cushion it. An elastic stocking with holes for the 4 legs was placed over the bandages, covering the pig from neck to tail. The stocking and bandages were changed every second day. To date this approach has worked well.

Since continuous contact of the native dermis with the cell-coated surface during the initial healing period is considered a necessary condition for successful PAD implantation, a technique had to be developed for excising a circular area of skin of precisely specified diameter. A carbide trephine was finally developed for this purpose.

To implant the PAD, its exact dimensions were drawn on the skin surface. A 10-12 cm lateral skin incision, 2 cm cephelad from the edge of the desired pocket, was made down to the level of the hypodermis. A 10 cm diameter pocket immediately deep to the dermis and superficial to the underlying hypodermis was made by sharp dissection. In the first 6 attempts, the epidermis was then removed from a circular region 6 mm larger in diameter than the PAD neck. Next, the skin over the pocket was stretched and a circular hole to accommodate the neck of the PAD cut using the carbide trephine. In the last 7 implants, a radial incision was made from the hole to allow stretching during the introduction of the PAD. The assembled PAD was inserted into the pocket and the neck of the device guided carefully through the circular opening so as not to shear the fibroblast culture. The incisions were closed with interrupted 2-0 nylon monofilament sutures.

RESULTS AND DISCUSSION

Preparation of PAD Neck Materials for In Vitro Cultivation of Fibroblasts. As reported previously, we are able to control the dimensions of pores created in the selected biomaterials. By varying the time and temperature of etching in NaOH, pores ranging from 0.5 to 5.0 μ in diameter are produced. By varying the time of nuclear bombardment, densities ranging from 15,000 to 30,000 pores/mm^2 have been obtained. We are currently investigating the biological effects materials with different pore dimensions and densities have on surrounding tissue after implantation.

Developing the Complete ALCON Surface. Early observations indicated that application of HFN, 4 ng/ml, to the Lexan substrate speeded up cell growth[1]. However, it was later observed that a more intimate and continuous apposition to the plastic substrate resulted when HFN pretreatment was omitted. This is clearly seen when TEM's of fibroblasts growing on nanoporous Lexan with no HFN pretreatment (Figures 1 and 3) are compared with a TEM of fibroblasts growing on a similar substrate after pretreatment with HFN as reported earlier[1]. Not only was the attachment to the surface of the Lexan inhibited by the HFN, but no contact with the pores was observed. However, both Figures 1 and 3 show close cell-to-substrate contact. Also, cell processes tended to fill the pores completely in the absence of HFN, even when the pores were very deep (Figure 3). Subsequent preparation of PAD neck material for fibroblast cultivation did not include HFN pretreatment as a result of these observations.

Bonding of Autologous Cells to Host Dermis. Completed in vivo experiments performed in rats to study the ability of the PAD, coated with autologous cells and collagen to inhibit epidermal migration, can be summarized by observing the representative histological sections shown in Figures 4 and 5. All implants showed signs of normal wound healing although the rate was slowed considerably by the substantial removal of dermis as a result of the surgical procedure used to remove surrounding epidermis. The tissue immediately surrounding the implants showed hyperepithelialization, large amounts of neutrophils in the dermis and remnants of scab. The implants of ALCON surface PAD's usually resulted in epidermal migration up to but not down the neck of the implant (Figure 4). This histological section represents ALCON surfaced Lexan implanted for 187 days. Figure 5 is a representative histological cross section of a smooth surfaced control PAD implanted for 72 days. The epidermis appeared to be moving down the wound opening. This was the result in most of the control implants. However,

it must be noted that in both experimental and control groups, wound healing at the implant site was not complete at the time of excision.

In Vivo Assessment in Miniature Swine. The results of 13 PAD implantations into swine are summarized in Table I. In control implants, a gap between neck and surrounding tissue tended to form about 4 wks postoperatively. Coated implants, on the other hand, appeared to maintain close tissue apposition to the PAD neck in 6 of 10 cases, as judged by gross examination (Figure 6). Only one animal has been sacrificed thus far, and the histologic results are as yet unavailable.

In these experiments the hypothesis that the flange shields the tissue-PAD interface[1] from applied forces received additional confirmation. In one instance a swine succeeded in bending the flange's steel reinforcing plate, but the tissue-device interface remained intact.

TABLE I. STATUS OF SWINE WITH FULL-SCALE PAD IMPLANTS

Pig No.	Implant: Age (wks)	Implant: Nanoporous (N) Smooth (S)/ Coated Neck	Epidermis (Amount Removed/Radial Incision)*	Postoperative Observations: Fluid Accumulation	Postoperative Observations: Infection (Postop Wk Detected)	Postoperative Observations: Edema[X]	Gap Around PAD Neck
156-1	48	(S)/No	5 mm/No	Yes[+]	-	+	1 mo; widening
157-1	29	(N)/Yes	3 mm/No	Yes[#]	28.5	+++	-
	28	(S)/Yes	3 mm/No	Slight	22.5	+	Slight at 10 wks[Z]
157-8	31	(S)/No	3 mm/No	No	-	+	4 wks
	30	(S)/No	3 mm/No	No	-	+	4 wks
159-3	24	(N)/Yes	3 mm/No	No	-	+	23 wks
154-10	20	(N)/Yes	0/Yes	No	-	+	Slight at 4 wks
	5	(N)/Yes	0/Yes	No	-	+	-
144-2	9.5	(N)/Yes	0/Yes	No	-	+	8.5 wks
	4.5	(N)/Yes	0/Yes	Yes[&]	[@]	++	-[V]
150-4	8	(N)/Yes	0/Yes	No	-	+	-
	5	(N)/Yes	0/Yes	No	-	+	-
33-3	3.5	(N)/Yes	0/Yes	Slight	-	+	-

*Hydron burn dressing used in first 7 implants.
[+]Under flange; decreased gradually.
[#]May be due to venous congestion secondary to doughnut-shaped pressure bandage used first postop wk
[&]Substantial during first 2 wks.
[@]Infection at incision as result of necrosis at edge of flange due to incorrect surgical placement.
[X]+ = slight; ++ = moderate; +++ = substantial.
[Z]Animal sacrificed at 29 wks.
[V]Implant removed at 4.5 wks.

CONCLUSIONS

Several tentative conclusions appear warranted at this stage of our research:

1. Fibroblasts cultivated in vitro under suitable conditions attached to nanoporous surfaces of Lexan 104 and silicone polycarbonate, forming a dynamically stable multilayer of living cells. This coating of autologous cells and collagen appeared to carry on physiological processes of metabolism following implantation.

2. Epidermal migration appears to halt at the tissue-device interface in the limited number of specimens thus far available for histologic evaluation. There are suggestions, but as yet no proof, that the cultured PAD surface and the host dermis merge, and hence that normal physiologic mechanisms account for inhibition of epithelial downgrowth. The data are as yet insufficient to allow predictions as to the duration of such inhibition.

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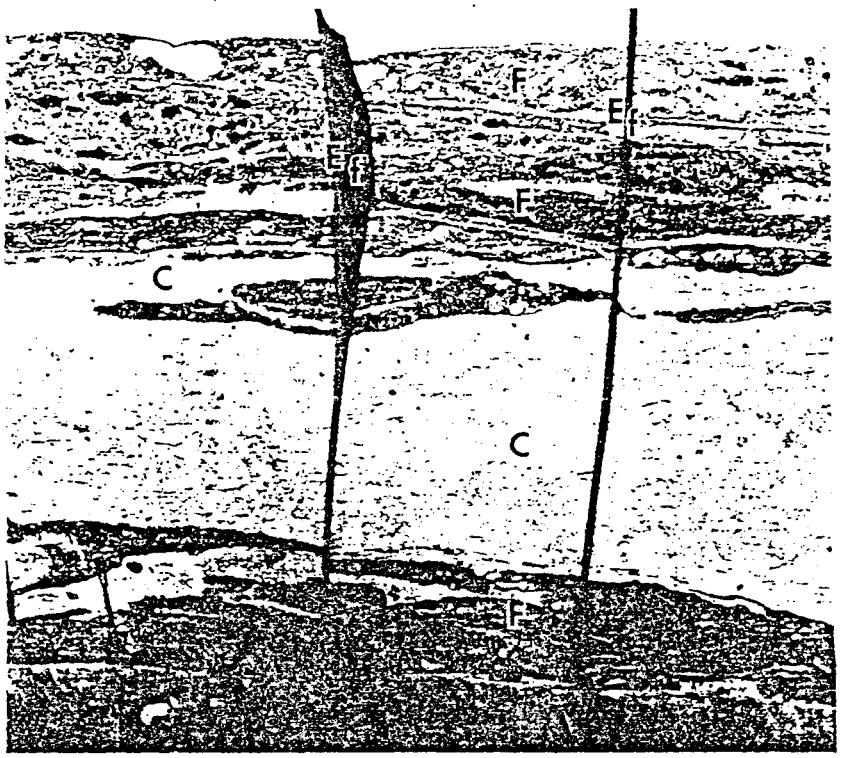

Figure 1. TEM of rat fibroblasts on a nanoporous Lexan substrate (L). No pores are visible. Numerous fibroblast layers are apparent (F) as well as abundant amounts of collagen (C). The lowest fibroblasts layer appears to be tightly bonded to the substrate. Folds were artificially created as a result of sectioning the embedding material (Epon) and plastic substrate due to different consistencies (E_f). (Reduced from original magnification of X7,500).

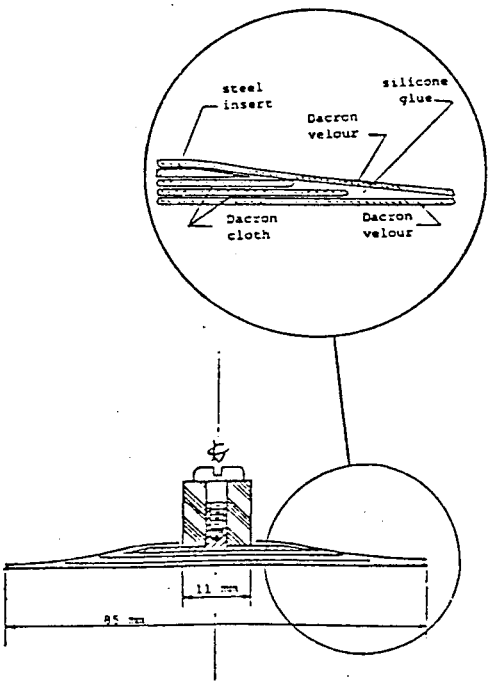

Figure 2. Simplification section of the full-scale PAD. Inset shows internal construction detail. Vertical dimensions of flange are out of scale.

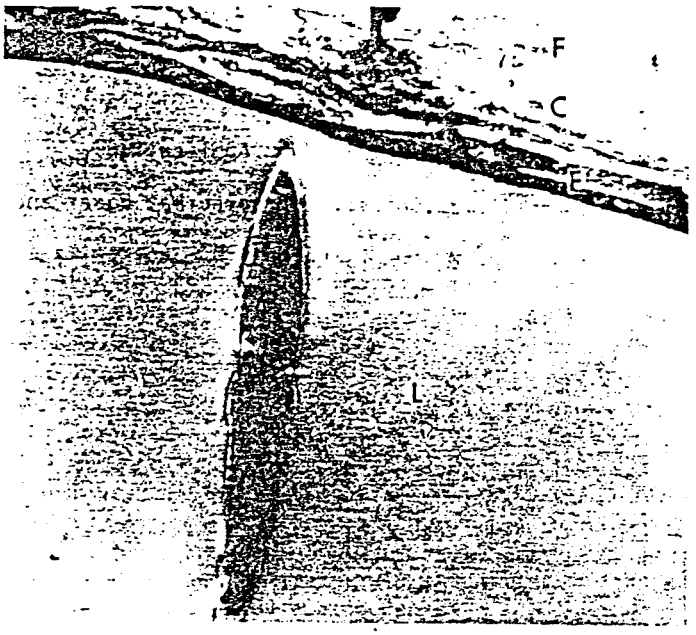

Figure 3. TEM of rat fibroblasts (F) on a nanoporous Lexan substrate (L). A very deep pore is seen here, completely filled by a process from a fibroblast on the Lexan surface. The pore is not shown as extending to the surface because of the angle of the section. Note the close association between the fibroblasts and the Lexan surface. The pore diameter is approximately 1 μ. (3 wks after start of subculture, reduced from original magnification of X10,000).

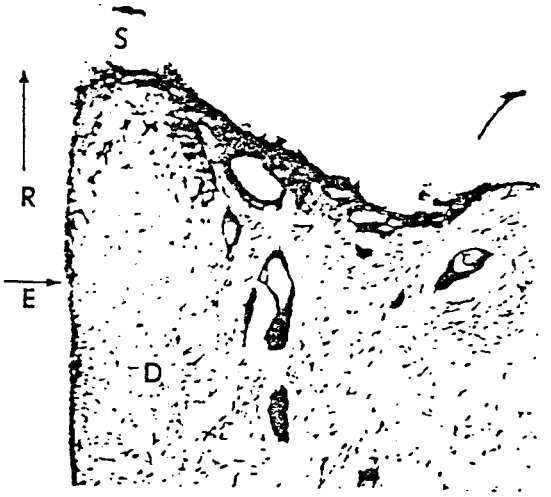

Figure 5. Photomicrograph of section from control Lexan rod (nonporous and not coated with fibroblasts) 72 days after implantation. The remnants of a scab may be seen (S) and a large number of neutrophils is present in the dermis (D). E = furthest point of epidermal migration. Notice that the epidermis has started to move down the wound opening. Most likely, this would eventually have led to marsupialization. (Hematoxylin and eosin, reduced from original magnification of X240).

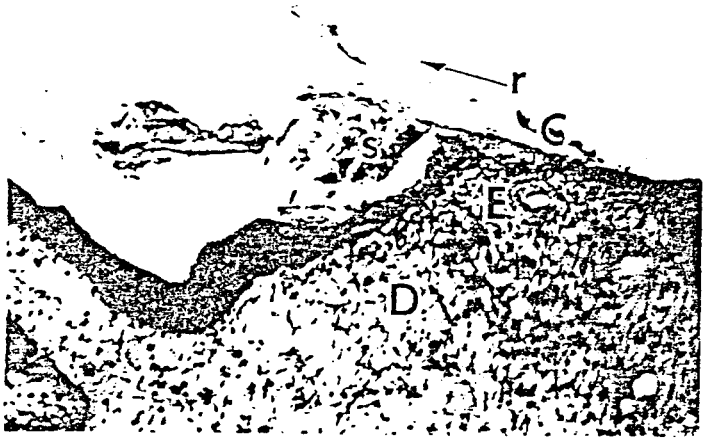

Figure 4. Photomicrograph of section from nanoporous coated rod 187 days after implantation. R = rod (arrow points away from rat); E = furthest point of advance of basal cells; S = scab remnant; D = dermis. Note that epithelial advance has come to a halt at dermis-device interface. Gap between dermis and rod is preparation artifact. Cell remnants define proximal surface of rod in region of gap. (Hematoxylin and eosin, reduced from original magnification of X140).

Figure 6. Photograph of nanoporous, coated PAD 30 days postimplantation in Swine 157-1. (Reduced from original magnification of X5).